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Response of artificial human skin to irritants: cytokine and prostaglandin release

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ABSTRACT

Cytokines have been implicated in aspects of vesicant injury/repair. This study describes responses of artificial human skin (Skin^2 and EpiDerm) to chloroethyl ethyl sulfide (CEES), defined by interleukin-1α (IL-1α), tumor necrosis factor-α (TNF-α) and prostaglandin E_2 (PGE_2) release. Skin^2 and EpiDerm in Millicells of 6 well Costar trays containing 1ml of assay media/well were exposed to CEES (2.0mg/L, flow rate 1L/min for 2h) in humidified air. Control tissues were exposed without CEES. Millicells containing Skin^2 or EpiDerm (12/group) were transferred to fresh assay media and incubated for 22h. Tissues (6/group) were used for MTT tests. Media from each well were stored in liquid N_2. IL-1α (RIA or ELISA), PGE_2 (RIA or EIA), and TNF-α (EIA) were measured in thawed specimens. CEES significantly increased release of IL-1α (192pg/ml ± 34.9, control 52pg/ml ± 16.6) and PGE_2 (3,977pg/0.1ml ± 1,197, control 2,541pg/0.1ml ± 574) from Skin^2, but not TNF-α levels, with viability (MTT) 3%. Neither IL-1α nor TNF-α were elevated by CEES-exposed EpiDerm, although PGE_2 was elevated (258pg/0.1ml ± 71 vs 184 ± 79), viability 46%. We conclude pro-inflammatory mediators, IL-1α and PGE_2, could play significant roles in CEES injury and that either fibroblasts are critical to the process, or EpiDerm, which lacks fibroblasts, is somehow more resistant.

INTRODUCTION

Sulfur mustard (HD), a vesicant reportedly used in recent regional conflicts, is a product with high potential as a military threat due to its low cost, high availability, and effective debilitating capacity. The pathology of HD injury includes damage to epithelial cells and to deeper cells initiating an acute inflammatory response, serum leakage, leukocyte infiltration, fibroblast activation, microblister formation in humans, and epithelial necrosis (6). Braue (1) described intracellular edema, basal cell necrosis, microblister formation, and follicular necrosis in the hairless guinea pig model for human injury. Electron microscopy (13) indicated widening of intercellular space at the basal cell level, loss of desmosomal attachments, cell rounding, nuclear condensation and pyknosis, rearrangement of tonofilaments to a perinuclear position, perinuclear blebbing, vacuolization, dilation of endoplasmic reticulum, electron densities (opacities), and necrosis with Testskin. Petrali (14) also indicated that human lymphocytes and keratinocytes show similar responses in vitro to those of human skin; however, isolated cells may be more sensitive than skin. Although extensive research defines biochemical and histological events in diverse models (2,4,7,8,9,10,11, 15), aspects of the mechanisms of action and unequivocally effective preventive and therapeutic measures are lacking.

Only limited information is available on the effects of vesicants on inflammatory products such as cytokines and prostaglandins which can be released from injured skin cells or from cells circulating through the exposed area in blood. Monteiro (12) detected HD-induced prostaglandin E_2 (PGE_2) release from porcine skin flaps, and Dannenberg (5) observed HD-induced cytokine gene expression (interleukin-1β (IL-1β), interleukin-8 (IL-8), monocyte chemo-attractant protein-1 (MCP-1) and growth stimulating chemo-attractant (GRO)), by in situ hybridization, after exposure of rabbit skin to HD. In vitro studies by Wilmer et al. (16) also supported the potential contribution of keratinocytes to chemically-induced inflammatory response. In his work increases in intracellular IL-1α and IL-8 secretion were the most prominent. Since these reports indicate prostaglandins and cytokines are products of HD exposure, their role in injury/repair should be determined. This study describes responses of artificial human skin (Skin² and EpiDerm) to chloroethyl ethyl sulfide (CEES), defined by IL-1α, tumor necrosis factor-α (TNF-α), PGE_2 and tetrazolium (MTT) viability responses.

METHODS

Artificial Human Skin (Skin²):

Skin² was purchased from Advanced Tissue Sciences Inc., La Jolla, CA. This skin is delivered in a 24-well sealed carrier tray (24 specimens), along with four millicell plates, maintenance media and assay media (which is used for short-term experiments such as these conducted in a CO_2 incubator). Each specimen of Skin² is an approximately 11 mm square by 1mm thick layer of differentiated epidermal (keratinocytes) and dermal (fibroblasts in collagen) human cells with a nylon support membrane embedded in the dermal layer. All specimens were incubated overnight in maintenance media before use. In this study 1.0ml of assay media was placed in each of the six wells of sterile assay trays and two trays (12 specimens per group) were exposed to humidified N_2 carrier gas, CEES in N_2 carrier gas (1.88.0mg/L, 1L/min for 2h or 225.6mg total vapor exposure), humidified air carrier gas or CEES in air carrier gas (2.0mg/L, 1L/min for 2h or 240mg). After exposure, tissues were placed on fresh assay media and incubated at 37°C in a CO_2 incubator for 22h. Media was stored in liquid nitrogen for later analyses and tissues were processed for MTT viability tests or microscopy.

Artificial Human Skin (EpiDerm):

EpiDerm was purchased from MatTek Corp., Ashland, MA. This tissue, highly differentiated human epidermis in 10mm diameter millicell wells, was refrigerated overnight. The next day, the millicells were transferred to 1ml of assay media, incubated for 1h and exposed to either humidified air carrier gas or CEES in air carrier gas. After exposure, tissues were placed on fresh assay media and incubated at 37°C for 22h. Media was stored in liquid nitrogen for later analyses and tissues were processed for MTT viability tests and microscopy.

IL-1α:

Assays were performed using either RIA (PerSeptive Diagnostics, Cambridge, MA) or ELISA BioTrak (Amersham Corporation, Arlington Heights, IL) as described in Company literature. Levels detected in specimens from controls were compared to those of treated groups.

PGE_2:

Assays were performed using either RIA (PerSeptive Diagnostics, Cambridge, MA) or EIA (Amersham Corporation, Arlington Heights, IL). Assays were performed as described in Company literature.

TNF-α:

Assays were performed using Biokine EIA (T Cell Diagnostics, Cambridge, MA). Assays were performed as described in Company literature.

MTT Viability Assay:

The MTT viability test kits were obtained from Advanced Tissue Sciences and MatTek Corp. In this test, viable cells reduce MTT to form an insoluble formazan precipitate which is extracted and quantitatively determined by photometric assay. The concentration is proportional to the relative number of viable cells. Assays were performed as described in Company literature.

Statistics:

Student's T-Test was used to compare treated and control groups ($p<0.05$).

RESULTS AND DISCUSSION

Exposure of Skin[2] to 225.6mg of CEES vapor in N_2 carrier gas, over a two hour exposure period, resulted in significantly elevated IL-1α release (Table 1). A similar

Table 1
IL-1α released over 22 hour period after a 2 hour CEES exposure.

	N_2 carrier gas	Air carrier gas	MTT % viability
Control	911 ± 198	52 ± 17	100
CEES treated	2,346 ± 484*	192 ± 35*	3** viability

* Mean values (pg/ml ± SD, T-test, $p<0.05$) for treated groups were significantly higher than controls. MTT mean values expressed as % of control, ** significantly lower than control value.

phenomenon occurred when air was used as the carrier (240mg of CEES vapor), although hypoxia increased both control and treated values. The release of PGE_2 followed a similar pattern to that of IL-1α (Table 2). However, IL-1α levels were increased by 3.7 times control values while PGE_2 levels were only increased by 1.6 times control values. This may indicate a more critical role for IL-1α, particularly if the bioactivity level and potential for amplification are also greater for the cytokine.

Table 2
PGE_2 released over 22 hour period after a 2 hour CEES exposure.

	N_2 carrier gas	Air carrier gas
Control	5,295 ± 1,011	2,541 ± 574
CEES treated	7,695 ± 2,983*	3,977 ± 1,197*

* Mean values (pg/0.1ml ± SD, T-test, $p<0.05$) for treated groups were significantly higher than controls.

CEES did not induce release of TNF-α in this model (Table 3), although the cells are capable of TNF-α release as indicated in methyl salicylate-treated and control Skin[2] data from another study (89.12pg/ml vs 78.69pg/ml).

Table 3
TNF-α released over 22 hour period after a 2 hour CEES exposure.

	N_2 carrier gas	Air carrier gas
Control	5.93 ± 1.83	7.46 ± 1.11
CEES treated	0.63 ± 1.18*	5.15 ± 0.82*

* Mean values (pg/ml ± SD, T-test, $p<0.05$) for treated groups were significantly lower than controls although all values were low.

EpiDerm, which contains keratinocytes but no fibroblasts, was used in these studies to determine which cell types were responsible for release of IL-1α and PGE_2 and to improve the sectioning qualities. The keratinocyte layer easily separates from the membrane with this product.

Table 4
IL-1α, PGE_2 and TNF-α released over 22 hour period after a 2 hour CEES exposure for EpiDerm.

	IL-1α	PGE_2	TNF-α	MTT
Control	65 ± 26	184 ± 79	0.5 ± 1.7	100
CEES treated	68 ± 31	258 ± 71*	3.0 ± 7.0	46**

Mean values for treated and control groups (pg/ml ± SD for IL-1α and TNF-α, pg/0.1ml for PGE_2; T-test, $p<0.05$).
*Significantly higher, ** significantly lower than control.

Only PGE_2 levels were elevated by CEES treatment with this model, although viability was reduced to 46% (Table 4). Conventional histology with H & E stained paraffin sections of Skin² indicated CEES-induced separation of the dermal and epidermal layers, but no discrete blister formation. No attempt was made to detect changes in adhesion molecules in this area.

Conclusions

Human skin cells in Skin² and EpiDerm can release all three pro-inflammatory mediators which were measured in this study (IL-1α, PGE_2, TNF-α). However, in these two models, CEES-induced release of TNF-α from skin cells (keratinocytes or fibroblasts) appears not to be a primary mechanism. This would not preclude initiation of a TNF-α response from other cell types present in vivo. The CEES-induced release of IL-1α from Skin² implicates keratinocytes/fibroblasts as primary or initial sources for this cytokine in CEES injury. The absence of a CEES-induced IL-1α response with EpiDerm could indicate a mechanism requiring communication between keratinocytes and fibroblasts. An alternative conclusion is that EpiDerm is more resistant than Skin² to CEES. Yet, the higher viability observed with EpiDerm could also be attributed to the absence of fibroblast-keratinocyte communication. With EpiDerm the PGE_2 response to CEES, in the absence of an IL-1α response, suggests that these are independent events. However, regulatory feed-back between these mediators does occur.

Zhang et al. (17) observed HD-induced release of both IL-1α and PGE_2 from an elegant perfused porcine skin model. The fact that both the human artificial skin model and the porcine skin flap model implicate these pro-inflammatory mediators in vesicant injury tends to substantiate their role in injury/repair. The latter model eliminates the requirement for blood components, although it includes other cell types such as endothelial and smooth muscle cells. The artificial skin model elicits this response from keratinocytes and fibroblasts. Dannenberg (5) detected mRNA for Il-1β with HD treated rabbit skin, but he did not measure IL-1α message. Data on the latter would permit distinction between gene activation and release of preformed IL-1α. Analyses after cell lysis or immunohistochemical localization could serve the same purpose. Since hypoxia amplified release of pro-inflammatory mediators, ischemia may have a similar effect. These results indicate that each model (cell, tissue, organ and animal) should provide specific aspects to resolving the mechanisms of vesicant-induced injury/repair and determining the efficacy of vesicant antagonists.

We did not attempt to monitor soluble receptors or receptor antagonists in these studies, but they should be a part of future experiments.

DISCLAIMER

The views, opinions and/or findings contained in this abstract are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation. Citations of commercial organizations and trade names do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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